METHODS FOR DETERMINING THE PRESENCE OF BRAIN PROTEIN S-100

The present invention relates methods for diagnosis and follow-up of patients with cerebral dysfunction as well as melanoma cancer, by determining the presence of the

5   brain protein S-100. The invention also relates to peptides comprising useful antigenic determinants from S-100 as well as monoclonal antibodies binding to these peptides.

As is known, the nervous system contains a number of proteins unique to its various

10   cellular elements. The cellular disruption of nervous tissue and cells of neural origin, by any pathogenic process, trauma or by neurological diseases, results in the release of normal soluble endogenous cytoplasmic proteins into the cerebral extracellular fluid and ultimately to other body fluids including the cerebrospinal fluid (CSF) and blood (serum and plasma). Examples of representative soluble small molecule

15   weight proteins of this type can be found in the S100 protein family. A review of this family can be found in Zimmer et al., Brain Research Bulletin, Vol. 37, pp 417-429, 1995.

Following disruption of cell membranes, these proteins are released into the

20   extracellular fluid in accordance with a time course and in quantities relative to the pathogenesis of the disease process or the extent of the brain tissue damages. The proteins diffuse into the CSF and then the blood or directly into the blood. The above mentioned cell membrane disruption is reflected by the blood plasmaor serum levels of one or more of these antigens and markers. These protein antigens have the

25   advantage of being stable and specific, not only for the brain, but for the cellular components in the brain. By following the relative release of the various nervous system protein antigens, it is possible to deduce the kind of destructive process occuring in the course of neurological diseases and/or the extent of possible brain tissue damages. Information of this type permits the diagnosis, evaluation of severity

30   and rate of progression of the above mentioned dieases and damages.

It is previously known to determine the amount of S-100 polypeptides in a clinical sample. US-A-4 654 313 discloses a radioimmunological assay method for S-100

protein. The patent document does neither mention anything about different kinds of S100-polypeptides nor about on which epitopes the assay method is based. The detection limit is declared to be 0.20 ng/ml but concentrations between 1.5 and 2.5 ng/ml is required in order to have less than 10% false positives. This concentration is

5   rather high. Moreover, in some countries it is not permitted to use radioactive methods in clinical assays.

It is also known to determine S-100 polypeptides by using ELISA-related methods. GB-A-2 109 931 discloses a solid-phase immunoanalysis method comprising the use

10   of enzyme-labelled antigens and particles coated with protein A on which antibodies are bound. S-100 proteins are only mentioned in claim 8 and nothing is revealed about the sensitivity of the method.

JP-A-6/109 734 describes a method suitable for analysing S-100 polypeptides, using

15   a first polyclonal antibody fixed to magnetic particles, and a second labelled polyclonal antibody. The method requires two different enzymes, namely horseradish peroxidase and alkaline phosphatase, and it comprises at least ten consecutive steps. The minimum detection limit is stated to be 0.02 ng/ml for cerebrospinal fluid and 0.06 ng/ml for bovine brain.

20
The complexity of clinical samples is often a serious problem. An assay method may give excellent results with artificial samples in the laboratory but quite a number of unreliable results might be obtained when the method is tested under clinical conditions. When it comes to immunological assays the problems are often caused

25   by an improper selection of antigenic determinants. One antibody in an assay comprising the use of two different antibodies, may be a hindrance to the other antibody when bound to the antigen to be determined. An improper selection of epitope for an antibody involved in the detection process may result in that the detection goup is completely or partially embedded in a protein complex and not av-

30   ailable for detection. Different proteins present in the sample might interfere. Moreover, a method comprising many consecutive steps may give uncertain results for complex clinical samples. as the interference possibilities increse with the number of steps and added extra components.

There is always a need for improvment of methods for analysing substances of medical intrest in clinical samples. An ideal clinical assay method should be quick, accurate and possible to perform with all types of clinical samples without

5   degeneration of the accuracy for certain types of specimen. It should also require a minimum of extra components. This applies to determination of S-100 polypeptides as well as other substances of medical interest.

Summary of the invention

10

Now it has turned out that by using antibodies directed to epitopes in the region from ser1 to asn38 and from thr82 to glu93 of the amino acid sequence of human S-100β polypeptide, an improved clinical assay method for determining S-100 polypeptides and particularly the β subunit or isoform thereof is obtained. Hence, the main object

15   of the present invention is an assay method using monoclonal antibodies directed to these epitopes. Another object of the present invention relates to short peptides having sequences corresponding to parts of the amino acid sequence of the human S-100β polypeptide from ser1 to asn38 and from thr82 to glu93. Yet another object of the present invention relates to analytical kits for carrying out the assay methods.

20

Detailed description of the invention

As already mentioned above it is often very difficult to outline methods for analysing clinical samples. It is neccesary that the method has a high sensitivity and

25   gives accurate results. It is also very important that known and unknown constituents of the sample other than the analyte do not influence the results. The present invention relates to an immunological assay method for determining the presence and/or content of human S-100 polypeptide based upon a selection of suitable S-100 epitopes and corresponding antibodies which fulfil the above mentioned

30   requirements.

It has turned out that the selected epitope combinations provides tests and test kits where:

1. a high sensitivity is achieved;

2. the antibodies of the kit binds equally strong to the internal standard as to the analyte in the clinical sample;

3. the epitopes are chosen in such a way that the different antibodies do not interfere with each other when they bind to the analyte, i.e. that the epitopes are situated sufficiently distant from each other.

The epitopes of the present invention are all comprised in the human S-100β polypeptide. Epitopes present within the amino acid sequences:

SELEKAMVALIDVFHQYSGREGDKHKLKKSELKELINN (SEQ. ID. NO. 2) and

TACHEFFEHE (SEQ.ID.NO. 3)

are preferred. Particularly preferred are epitopes comprised within the peptide

AMVALIDVFHQYSGREGDKHKLKKSELKELINN (SEQ. ID. NO. 4) and

especially within the peptides REGDKHKLKKSELKEL (SEQ. ID. NO. 5) and

EFFEHE (SEQ. ID. NO. 6).

The disclosed epitopes are, among all, used to construct peptides for inducing the formation of suitable antibodies on which the claimed assay method is based. These peptides mostly consist of up to 38 amino acids. The whole amino acid sequence of a peptide according to the present invention is derived from human S-100β. These peptides may comprise variants wherein the original amino acid sequence is modified or altered by insertion, addition, substitution, inversion or deletion which preferably show at least 90% homology with the sequence of SEQ. ID. NO. 2 and SEQ.ID.NO.3 and retain essentially the same immunological properties. The peptides may also comprise multiples of certain epitopes, and in this case their sequence length may exceed 38 amino acids.

By the expression "sub-fragment" is meant a polypeptide sequence having a length of at least 6 amino acids.

5   The epitopes can also be used to construct fusion peptides comprising at least two distinct epitopes which, among all, can be used as internal standard in immunoassays.

<u>Abbreviations</u>

10   The following abbreviations are used:

	S100	-S100β
	RT	-Room Temperature
	BSA	-Bovine Serum Albumin
15	Mab(s)	-Monoclonal antibody(ies)
	kD	-kiloDalton
	ECL	-Enhanced Chemiluminescent Assay
	CBB	-Commassie Brilliant Blue
	LIA	-Luminometric Immuno Assay
20	IRMA	-Immuno Radio Metric Assay
	ELISA	-Enzyme Linked ImmunoSorbent Assay
	SDS-PAGE	-SodiumDodecylSulfate - PolyAcrylamideGelElectrophoresis
	PBS	-Phosphate Buffered Saline
	RLU	-Relative Light Units
25	NHS	-N-HydroxySuccinimide
	EDC	-N-ethyl-N'-(dimethylaminopropyl)-carbodiimide
	RAMFc	-RabbitAnti-MouseFc antibody
	EDTA	-EtylenDiamineTetraAcetic acid
	NaCl	-Sodium Chloride
30	NaN$_3$	-Sodium azide
	iv.	-intravenously
	aa	-amino acid

ng   -nanogram

ml   -millilitre

mg   -milligram

HRP   -HorseRadish Peroxidase

5   h   -hour(s)

min   -minute(s)

sec   -second(s)

Experimental details common to all test procedures

10

The peptides were prepared by the methods disclosed in Merrifield (1963), J. Am.
Chem. Soc., vol. 85, p 2149; Gutte et al.(1971), J. Biol Chem vol. 246, p. 1922; and
Carpino et al. (1970), J Am Chem Soc vol. 92, p. 5748.

15   The monoclonal antibodies were prepared by the method according to Köhler et
al.(1975), Nature vol. 256, p. 495; and Harlow et al.(1988), Antibodies. A
Laboratory Manual, Cold Spring Harbor, p. 139.

Antigen and Standard preparations

20

Procedure for preparation and purification of S100 antigen prior to immunisation of
Balb/c mice was according to Moore (Biochim. Biophys. Res. Comm. 1965, 19: 739
- 744) with a slight modification according to Haglid & Stavrou (J. Neurochem.
1973, 20:1523-1532). Briefly, bovine brain was homogenised in Tris buffer, pH 7.2.
25   The homogenate was centrifuged at 10.000 r.p.m. and the clear supernatant was used
for further purification by ammonium sulphate precipitation. The fraction still
soluble after saturation by ammonium sulphate was dialysed and purified by
separation on a Sephadex G150 Sepharose (Pharmacia Biotech AB, Uppsala
Sweden)chromatographic column followed by separation on a DEAE-sephadex
30   (ionic exchange) column (Pharmacia Biotech AB, Uppsala Sweden). The fraction
eluted by 0.3 - 0.4 M NaCl was collected, desalted, lyophilised and used for further
experiments.

Hybridoma construction.

Balb/c mice were immunised with purified S100ββ intraperitonially in Freund's
complete adjuvant and were given booster iv. injection 6 weeks later during 3
5   consecutive days. The spleen was removed on the fourth day after last injection and
prepared for fusion. The myeloma cell line Sp2/0-Ag14 was used for fusion of
Balb/c spleen cells.

Antibody purification and subclass determination.

10

Monoclonal antibodies were identified, extracted and purified from hybridoma
supernatant according to Harlow & Lane Eds. in ANTIBODIES A LABORATORY
MANUAL, Cold Spring Harbour Laboratory Press, New York 298-299 & 311.
Briefly, positive hybridoma clones carrying supernatant with specific antibodies
15   were identified using ELISA with microtitreplate wells coated with S100ββ.
Immunoglobulins were precipitated using saturated ammoniumsulphate and dialysed
against 1.5 M Glycine, 3 M NaCl, pH 8.9. Dialysed material were affinity
chromatography purified on an protein-A Sepharose (Pharmacia Biotech AB,
Uppsala Sweden) column. Fractions were neutralised by addition of small volumes
20   of 1M Tris pH 8.0.

Epitope mapping

S100β (monomer) epitopes for respective antibody was investigated by use of a
25   synthetic peptide library. Peptides were linked to nitro-cellulose filter membrane via
an amide link, according to the manufacturer (Research Genetics', USA) and covers
all ninety-one aa in the protein. In total the library consisted of thirty-one, all except
one being ten aa-residues long synthetic peptides. Each peptide was consecutively
shifted three aa towards the -COOH terminal end of the protein. Positive antibody-
30   binding was indicated by the use of a second anti-mouse antibody conjugated with
HRP and detected using an ECL assay (Amersham, UK).
Results:

Two binding sequences were found

Epitope 1

AMVALIDVFHQYSGREGDKHKLKKSELKELINN (residues 6-38)(SEQ. ID.

5   NO.4)

and

Epitope 2

10   EFFEHE (residues No 86-91) (SEQ. ID. NO. 6)

Antibody reactivity

15· Purified antibodies reacting with the epitopes were checked for reactivity and

     affinity using the BIAcore™ system (Pharmacia Biosensor AB, Uppsala Sweden).

     Briefly, in order to test the specificity of the antibodies, the RAMFc was

     immobilised onto the sensor chip CM5 NHS-ester activated surface, according to

     standard procedure, to provide approximately 600 RLU. Then each Mab was bound

20   to the RAMFc surface to approximately 300RLU, followed by the S100αα and the

     S100 standard (consisting of 50% S100αβ and 50% S100ββ) in separate

     experiments. All reactions were carried out in continuos flow of the phosphate

     buffer.. The kinetics between antibodies and antigen was done similarly . S100

     antigen was added to the chips at 200-450nM for reactivity measurements of the

25   antibody intended for the solid phase and at 1000-1500nM for measurements of the

     antibody intended for tracers. Kinetics was determined using the BIAcore™ Kinetic

     evaluation 2.1, software (Pharmacia Biosensor AB, Uppsala Sweden). It can be

     concluded from the reactivity profile that the antibodies reactive with the epitopes

     are specific for the β-containing forms of S100 and not the α-containing form.

30

Example 1

Development of an immunoluminometric procedure

5   Tracer antibody was conjugated with luminol. Briefly, ABEI (Sigma, St Louis, Ms)
was linked with a diactivated ester (Etylenglykolbis-succimidyl succinat, EGS). The
ABEI-EGS-conjugate was next mixed with monoclonal antiS100-antibody in an
approximately 50:5 molar ratio in 100µl of PBS pH 7.4, containing 15% acetonitrile
and incubated 1 h at room temperature. The ABEI-conjugated antibody was purified

10   on a Sephacryl®S 300 HR (Pharmacia Biotech AB, Uppsala Sweden) gelfiltration
column, and appropriate fractions were pooled and diluted in phosphate-buffer.

Preparation of antibody coated tubes for LIA.

15   Polystyrene tubes (Greiner, Germany) were incubated overnight at room temperature
with 3µg of S100-antibodies in 300 µl of PBS pH 7.5. The tubes were washed with
0.1% Tween20® in PBS. Next, tubes were blocked with a solution containing
0.9%BSA and 4% Saccarose and incubated for 24h. The solution was aspirated and
the tubes allowed to dry.

20

LIA test procedure.

The test was conducted in a two step procedure by incubating 100µl of patient body
fluid in antibody coated tubes, or S100 standard with 100 µl of diluent (PBS +

25   5%BSA) and incubated at room temperature. After washing 200 µl of the luminol-
labelled antibody was added and a further 2 h of incubation was performed before
measurement. After another washing the luminescence was developed using the
LIA-mat starter service kit (Byk-Sangtec, Diezenbach Germany) and immediately
measured as integrals over a period of 5 sec in luminometer (Berthold, Germany). In

30   order to convert the obtained light signal into concentration of S100 measurements
on patient samples were compared with measurements on solutions with known

concentrations of S100 (standards). The detection limit (zero standard +3 standard deviations) was approximately 0.01 µg/l.

<u>Preparation of a Standard curve</u>

S100B protein was obtained from Medisera, Lund, Sweden, and diluted in PBS + 5% BSA. Dilutions contained: 0.10, 0.40, 2.00, 8.00 and 20.00 µg/l, of an S100 preparation consisting of 50 % of the ββ form and 50 % of the αβ form. PBS + 5 % BSA was used as standard 0. Three measurements were carried out for each dilution. The measured results as well as statistical calculations are presented in table 1 below:

Table 1

Concentration	Counts	Average	Calculated conc.	Average
Standard 0	1996		0 µg/l	
	2024		0 µg/l	
	2053		0.0019 µg/l	
		2024		0 µg/l
0.10 µg/l	3142		0.135 µg/l	
	2760		0.0647 µg/l	
	2988		0.105 µg/l	
		2963		0.10 µg/l
0.40 µg/l	5494		0.394 µg/l	
	5620		0.405 µg/l	
	5579		0.401 µg/l	
		5564		0.40 µg/l
2.00 µg/l	21430		2.049 µg/l	
	21028		1.988 µg/l	
	20869		1.966 µg/l	
		21109		2.00 µg/l

Concentration	Counts	Average	Calculated conc.	Average
8.00 µg/l	68389		7.823 µg/l	
	67013		7.677 µg/l	
	74791		8.494 µg/l	
		70064		8.00 µg/l
20.00 µg/l	175560		22.12 µg/l	
	155141		18.54 µg/l	
	161052		19.51 µg/l	
		163918		20.00 µg/l

The lower detection limit was defined as three standard 0 determinations plus 3X the standard deviation value. For this measurement, it was calculated to be 0.006 µg/l.

Clinical determinations of S100 in serum

The S100 concentration was determined in serum from patients receiving heart bypass surgery and being connected to a heart-lung machine. The results are presented in table 2 below:

Table 2

Patient	Counts	Average	Concentration	Average
1	94698		10.61 µg/l	
	98104		10.99 µg/l	
		96401		10.80 µg/l
2	1716		Not detected	
	1478		Not detected	
		1597		Not detected
3	3762		0.23 µg/l	
	3799		0.23 µg/l	
		3780		0.23 µg/l

Patient	Counts	Average	Concentration	Average
4	13158		1.04 µg/l	
	14183		1.15 µg/l	
		13670		1.10 µg/l
5	8788		0.66 µg/l	
	8580		0.64 µg/l	
		8684		0.65 µg/l
6	10301		0.78 µg/l	
	10100		0.77 µg/l	
	10200			0.77 µg/l

Example 2

Development of an ELISA test procedure.

As tracer antibody was used monoclonal antiS100 antibody conjugated with β-galactosidase according to Harlow & Lane Eds. in ANTIBODIES A LABORATORY MANUAL. Cold Spring Harbour Laboratory Press, New York page 351.

Preparation of antibody coated microtiter wells for ELISA.

Microtiterplatewells (Corning, Denmark) were incubated overnight at +4°C with 2.5µg of microtiter wells were finally washed three times with 0.05% Tween20® and air dried before use.

ELISA test procedure.

The ELISA was conducted in a multiple step incubation procedure.
100 µl of 1:1 diluted patient sample or 100µl of S100 standard (0 - 20 µg/ml) was added to the wells.
The plate was incubated for 1.5h at RT under shaking.

The plates were washed three times with 300μl 0.05% Tween20® in PBS.

100 μl of alkaline phosphatase conjugated tracer antibody was added and a further
1.5h of incubation on a shaker was performed. ·

The wells were then washed three times with 0.05% Tween20® in PBS.

5   100μl of a 5% o-nitro-phenyl-β-galactoside substrate solution was added and the
plates were incubated with substrate for another forty-five minutes and colour is
developed.

The colour development was stopped by the addition of 100μl 0.66M Na_2CO_3.

10   Each well of the plate was read at 405nm in a standard microtiterplate reader. In
order to convert the obtained colour signal into concentration of S100, measurements
on patient samples were compared with measurements on solutions with known
concentrations of S100 (standards). The detection limit (zero standard +3 standard
deviations) was approximately 0.2 μg/l.

15   Result:

Standard (μg/l)	0	0.5	1.5	5	15
A 405	0.088	0.147	0.244	0.675	1.196

20

Example 3

Development of an immunoradiometric (IRMA) test procedure

25   IRMA tracer antibody conjugation

A monoclonal antiS100 antibody was conjugated with Iodine using the Chloramine
T method according to Greenwood et al. (Biochem. J. 1963, 89:114-123). The
specific activity was determined to be 520±80 MBq/mg

30

Preparation of antibody coated to polystyrene beads

Monoclonal anti S100 antibodies were coupled to polystyrene beads by the
Glutaraldehyde coupling method according to Harlow & Lane Eds. in ANTIBODIES,

5   A LABORATORY MANUAL, Cold Spring Harbour Laboratory Press, New York, 533 &
536-537. Final blocking was by 1% BSA, 0.1% NaN₃ in PBS pH7.5.

IRMA test procedure.

10   100µl of patient sample or standard was added to polystyrene tubes together with
100µl PBS diluent. One polystyrene coated bead was added to each tube followed
by incubation for 1 h at RT on a shaker. Next the beads were washed once with 2ml
of demineralised water and 200µl of I-125 labelled tracer antibody was added and
the tubes were incubated a further 2h on a shaker. After washing the radioactive

15   signal on the bead was measured in a standard γ-counter. In order to convert the
obtained radioactive signal into concentration of S100, measurements on patient
samples were compared with measurements on solutions with known concentrations
of S100 (standards). The detection limit (zero standard +3 standard deviations) was
approximately 0.1 µg/l.

20
Example 4

Use of IRMA test procedure for assay of S100 in serum from melanoma patients

25   The S100 based test procedure was applied on clinical questions relating to
melanoma. Blood samples from patients with melanoma of various stages of cancer
progression were collected in serum collecting tubes. Samples were then frozen and
treated according to the test procedure described above in Example 3.

30   Results:

Relationship to staging.

Clinical Stage I vs Clinical Stage II. In a study of 577 patients the geometric mean

for Stage I was found to be 0.12 µg/l and for Stage II the geometric mean was found

to be 0.33 µg/l.

p-value < 0.001.

Example 5

<u>Use of IRMA test procedure for assay of S100 in serum from melanoma patients</u>

The S100 based test procedure was applied on clinical questions relating to

melanoma. Blood samples from patients with melanoma of various stages of cancer

progression were collected in serum collecting tubes. Samples were then frozen and

treated according to the test procedure described above in Example 3.

Results:

Relationship to survival

Clinical Stage I vs Clinical Stage II and III. In a study with respect to survival

performed on 643 patients the relative hazard and 95% confidence interval was

calculated. The relative hazard was found to be 12.3 and the confidence interval 5.6-

27.2 with a p-value of <0.001

Example 6

<u>Use of the S100 LIA-method for evaluation of the influence of extra corporal</u>
<u>circulation equipment on the brain</u>

The S100 based test procedure in Example 1 was applied on monitoring cerebral

injury following extra corporeal circulation (ECC). Blood samples from patients

undergoing extra corporeal circulation were collected in serum tubes and treated

according " Test procedure". Results

	Before start of ECC	End of ECC	1 day after surgery	2 days after surgery
S100 levels µg/l	0	1,67	0,21	0,13

In this group of patients the level of S100 in serum was elevated for at least 2 days after surgery.

Uncomplicated cases should return to normal levels within the first 24 hours (Ref P. Johnson et al. J. Cardiothor .Vasc. Anaesthesia, 9:6 (1995) 694-99).

Example 7

Use of LIA test procedure for assay of S100 in serum from melanoma patients

The S100 based test procedure was applied on clinical questions relating to melanoma. Blood samples from patients with melanoma of various stages of cancer progression and blood donors were collected in serum collecting tubes. Samples were frozen and treated according to the test procedure described above in Example 1.

Result: Of 136 patients with various stages of melanoma 25 had a level of S100 below 0.08 and of 100 blood donors tested on the same occasion 7 had a level equal to or above 0.08 µg/l.

Example 8

The reliability of both the test and the S100β polypeptide marker per se when diagnosing melanoma were investigated. On 252 patients with melanoma, serum was drawn before treatment was started and determination of the level of S100β polypeptide was performed by the assay method disclosed in example 1. When a cut-off value of 0.16 µg/l was used, the medium survival time of patients having a S100β concentration above the cut-off value was 7 months, whereas the medium

survival time was more than 120 months for patients having a S100β concentration below the cut-off value.

In a patient diagnosed with malignant melanoma, considered to show no evidence of
5 disease and monitored by the immunoradiometric assay method as disclosed in example 3, elevated levels of S100β were recorded 2 months prior to the appearance of skin metastases and 6 months before metastases in organs were found.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

  (i) APPLICANT:
    (A) NAME: AB Sangtec Medical
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  (ii) TITLE OF INVENTION: Methods for determining brain antigens

  (iii) NUMBER OF SEQUENCES: 8

  (iv) COMPUTER READABLE FORM:
    (A) MEDIUM TYPE: Floppy disk
    (B) COMPUTER: IBM PC compatible
    (C) OPERATING SYSTEM: PC-DOS/MS-DOS
    (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

  (i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 91 amino acids
    (B) TYPE: amino acid
    (C) STRANDEDNESS:
    (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: protein

  (iii) HYPOTHETICAL: NO

  (iv) ANTI-SENSE: NO

  (v) FRAGMENT TYPE: internal

  (vi) ORIGINAL SOURCE:
    (A) ORGANISM: Homo sapiens

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

  Ser Glu Leu Glu Lys Ala Val Val Ala Leu Ile Asp Val Phe His Gln
  1   5   10   15

  Tyr Ser Gly Arg Glu Gly Asp Lys His Lys Leu Lys Lys Ser Glu Leu
            20   25   30

19

Lys Glu Leu Ile Asn Asn Glu Leu Ser His Phe Leu Glu Glu Ile Lys
         35   40   45

Glu Gln Glu Val Val Asp Lys Val Asn Glu Thr Leu Asp Ser Asp Gly
         50   55   60

Asp Gly Glu Cys Asp Phe Gln Glu Phe Met Ala Phe Val Ala Met Ile
65   70   75   80

Thr Thr Ala Cys His Glu Phe Phe Glu His Glu
              85   90

(2) INFORMATION FOR SEQ ID NO: 2:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 38 amino acids
      (B) TYPE: amino acid
      (C) STRANDEDNESS:
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

   (iii) HYPOTHETICAL: NO

   (iv) ANTI-SENSE: NO

   (v) FRAGMENT TYPE: N-terminal

   (vi) ORIGINAL SOURCE:
      (A) ORGANISM: Homo sapiens

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

   Ser Glu Leu Glu Lys Ala Met Val Ala Leu Ile Asp Val Phe His Gln
   1   5   10   15

   Tyr Ser Gly Arg Glu Gly Asp Lys His Lys Leu Lys Lys Ser Glu Leu
                  20   25   30

   Lys Glu Leu Ile Asn Asn
                  35

(2) INFORMATION FOR SEQ ID NO: 3:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 10 amino acids
      (B) TYPE: amino acid
      (C) STRANDEDNESS:
      (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
    (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Thr Ala Cys His Glu Phe Phe Glu His Glu
1   5   10

(2) INFORMATION FOR SEQ ID NO: 4:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 33 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS:
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (iii) HYPOTHETICAL: NO

    (iv) ANTI-SENSE: NO

    (v) FRAGMENT TYPE: internal

    (vi) ORIGINAL SOURCE:
        (A) ORGANISM: Homo sapiens

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

    Ala Met Val Ala Leu Ile Asp Val Phe His Gln Tyr Ser Gly Arg Glu
    1   5   10   15

    Gly Asp Lys His Lys Leu Lys Lys Ser Glu Leu Lys Glu Leu Ile Asn
                    20   25   30

    Asn

(2) INFORMATION FOR SEQ ID NO: 5:

    (i) SEQUENCE CHARACTERISTICS:

       (A) LENGTH: 16 amino acids
       (B) TYPE: amino acid
       (C) STRANDEDNESS:
       (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: peptide

  (iii) HYPOTHETICAL: NO

  (iv) ANTI-SENSE: NO

  (v) FRAGMENT TYPE: internal

  (vi) ORIGINAL SOURCE:
       (A) ORGANISM: Homo sapiens

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Arg Glu Gly Asp Lys His Lys Leu Lys Lys Ser Glu Leu Lys Glu Leu
1   5   10   15

(2) INFORMATION FOR SEQ ID NO: 6:

  (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 6 amino acids
       (B) TYPE: amino acid
       (C) STRANDEDNESS:
       (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: peptide

  (iii) HYPOTHETICAL: NO

  (iv) ANTI-SENSE: NO

  (v) FRAGMENT TYPE: internal

  (vi) ORIGINAL SOURCE:
       (A) ORGANISM: Homo sapiens

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Glu Phe Phe Glu His Glu
1   5

(2) INFORMATION FOR SEQ ID NO: 7:

  (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Asp Lys His Lys Leu Lys Lys Ser Glu Leu
1   5   10

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Lys Leu Lys Lys Ser Glu Leu Lys Glu Leu
1   5   10